



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: METHOD FOR INTRODUCING A CHEMICAL AGENT INTO THE SYSTEMIC CIRCULATION

## (57) Abstract

A method for introducing a drug, imaging agent and/or other chemical agent into the systemic circulation comprises providing a drug, imaging agent and/or other chemical agent in liposomes having a mean diameter of less than about 200 nm, preferably less than about 80 nm, as determined by negative-staining transmission electron microscopy. The liposomes are orally administered. It has been found that liposomes of such small sizes are not absorbed by the macrophages in the Peyer's patches of the gut, but instead are introduced into the venous circulation from the Peyer's patches, and then through the portal vein or inferior vena cava system, through the heart, and into the systemic circulation.

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## METHOD FOR INTRODUCING A CHEMICAL AGENT INTO THE SYSTEMIC CIRCULATION

### BACKGROUND OF THE INVENTION

Currently, a wide variety of chemical agents, such as growth hormone, insulin, and imaging agents, which require introduction into the systemic circulation must be administered parenterally. However, under certain circumstances, parenteral administration is undesirable. Accordingly, a need exists for a method for introducing such agents into the systemic circulation by oral administration.

### SUMMARY OF THE INVENTION

The invention relates to methods for introducing a drug, imaging agent and/or other chemical agent into the systemic circulation. The drug, imaging agent and/or other chemical agent is introduced into liposomes having a mean diameter of less than about 200 nm, preferably less than about 100 nm, still more preferably less than about 80 nm, and even more preferably less than about 50 nm, as determined by negative-staining transmission electron microscopy. The liposomes are orally administered. It has been found that liposomes of such small sizes are not absorbed by the macrophages in the Peyer's patches of the gut, but instead are introduced into the venous circulation from the Peyer's patches, and then through the portal vein or inferior vena cava system, through the heart, and into the systemic circulation. In contrast, it has been discovered that larger liposomes are taken up by the macrophages in the Peyer's patches and broken down in the macrophages. The invention provides a method for orally introducing into the systemic circulation chemical agents, and in particularly chemical agents that heretofore could only be introduced into the systemic circulation parenterally.

### DESCRIPTION OF THE DRAWINGS

Fig. 1 is an electron photomicrograph (magnification: 16,000) of liposomes in the cervical lymph tissue.

Fig. 2 is an electron photomicrograph (magnification: 16,000) of liposomes in the liver, which are partially lysed in comparison to the lyophilized liposomes visualized in saline before oral ingestion by the animals.

### DETAILED DESCRIPTION

In one embodiment, the invention is directed to methods for introducing a drug, imaging agent and/or other chemical agent into the systemic circulation. The drug, imaging agent and/or other chemical agent is introduced into liposomes having a mean diameter of less than about 200 nm, preferably less than about 100 nm, still more preferably less than about 80 nm, and even more preferably less than about 50 nm, as determined by negative-staining transmission electron

1 microscopy. The liposomes preferably have a mean diameter of at least about 20 nm. The liposomes are orally administered.

5 The most accurate method to measure the size of liposomes is direct observation by negative-staining transmission electron microscopy, particularly with liposomes less than 1 (one) micron in diameter. Individual liposomes can be viewed and the size and lamellarity (number of layers) can be viewed if they are lyophilized. The sizes can be determined as well as the range of sizes.

10 More specifically, negative staining is used to visualize viruses, cells, and large molecules, as well as other particles such as liposomes. The contrast in the transmission electron micrographs is provided by the osmium ion of the negative stain which scatters the electrons. Since liposomes are a series of phospholipid bilayers composed principally of carbon and hydrogen, relatively few electrons are scattered and the liposomes appear clearer than the negative contrast stain of the osmium ion. A suitable technique for negative-staining transmission electron microscopy is described in Haschmeyers, R.H. and Myers, R.S. Principles and Techniques of Electron Microscopy Biological Applications. (Hayat, M.A. editor, Van Nostrand Reinhold Co., New York) Vol. 2, 1972, the entire disclosure of which is incorporated herein by reference.

15 Exemplary agents that can be incorporated into the liposomes include any chemical agent that alters a bodily function or acts as an imaging agent which outlines a bodily structure, such as agents for ultrasound, the electromagnetic spectrum, x-rays of any sort, magnetic resonance imaging, positron emission, or electron beam scanning. The invention is particularly useful for agents that heretofore were capable of only being administered parenterally for introduction into the systemic circulation, and more particularly for those agents that are effective at relatively low doses, e.g., in the microgram range. Particularly suitable chemical agents include, but are not limited to, apomorphine, growth hormone, insulin, vascular endothelial growth factors (VEGF), platelet activation factors (PAF), cytokines, endothelial adhesion factors, sulfonylurea agents (hypoglycemia inducing), macrolide antibiotics, protein fusion agents of animal or yeast origin, slow channel inhibitors, angiotension converting enzyme inhibitors, herbs, alpha or beta sympathetic stimulating or inhibition agents, corticosteroids, male and female hormonal agents, such as estrogen, testosterone, and aldosterone, granulocyte stimulating factors, megakaryocyte stimulating agents, eicosanoids, diuretics such as furosemide, MRI and CT imaging agents, plasmids containing DNA or RNA fragments, genes or portions thereof, biguanides, anticholinergic agents, dopaminergic agents, anticoagulants, heparin, immunomodulators, antineoplastic agents, toxoids, thrombolytic agents, antiarrhythmics, contraceptives, antilipemic agents, vasodilators, vasopressors, dopamine receptor agents, somatostatin analogues, serotonin receptor agonists, reuptake inhibitors, ergot derivatives, antihistamines, antivirals, immunosuppressives, histamine receptor antagonists, smooth muscle relaxants, oxytocics, carbonic anhydrate inhibitors, xanthine derivatives, leukotriene antagonists, lung surfactants,

1 benzodiazepones, photo-sensitizing agents, sympatolytics, cytoprotective agents, antifungals,  
cerebral vasodilators, amebicides, analeptic agents, analgesics, aspirin, anxiolytic agents,  
salicylates, local anesthetics, angiotensin II receptor antagonists, anorectics, anthelmintics,  
chemo-therapeutic agents, antimalarials, antituberculosis agents, sulfonamides, leprostatics,  
5 anticonvulsants, antidepressants, anti-inflammatory agents, antiarthritics, antidiabetic agents,  
antihypertensive agents, biological response modifiers such as interferon, blood plasma fractions,  
antiplatelet agents, colony stimulating factors, iron, heparin antagonists, bone metabolism  
regulators, fertility agents, anti-inflammatory agents, gonadotropin releasing hormones and  
inhibitors, homeopathic preparations, mast cell stabilizers, nucleoside analogues, appetite  
10 suppressants, calcitonin, parasympatholytics and mimetics, antianxiety agents, radiopaque  
agents, steroidal anti-inflammatory agents, bronchodilators, antipsoriatic agents, smoking  
cessation aids, antispasmodics, weight control preparations, thyroid preparations, gadolinium and  
other paramagnetic magnetic resonance imaging agents, and gamma and beta radioisotope  
imaging agents.

15 The amount of chemical agent included in the liposomes depends on the particular  
chemical agent and the application for which it is to be used, as can be determined by one skilled  
in the art, and can range from 1 to 99 percent by weight of the liposomes.

The liposomes of the present invention may be made of any suitable phospholipid,  
glycolipid, derived lipid, and the like. Examples of suitable phospholipids include phosphatide  
20 choline, phosphatidyl serine, phosphatidic acid, phosphatidyl glycerin, phosphatidyl  
ethanolamine, phosphatidyl inositol, sphingomyelin, dicetyl phosphate, lysophosphatidyl choline  
and mixtures thereof, such as soybean phospholipids, and egg yolk phospholipids. Suitable  
glycolipids include cerebroside, sulphur-containing lipids, ganglioside and the like. Suitable  
derived lipids include choleic acid, deoxycholic acid, and the like. The presently preferred lipid  
25 for forming the liposomes is egg phosphatidylcholine.

The liposomes may be formed by any of the known methods for forming liposomes and  
may be loaded with a chemical agent according to known procedures. Known methods for  
forming liposomes containing chemical agents are described, for example, in U.S. Patent No.  
4,235,871 to Papahadjopoulos, et al., and Oral Microbiology and Immunology, 1994, 9:146-153,  
30 the disclosures of which are incorporated herein by reference.

Preparation of a homogeneous population may be accomplished by conventional  
techniques such as extrusion through a filter, the filter being either the straight path or tortuous  
path type. Other methods of treating liposomes to form a homogenous size distribution are  
ultrasonic exposure, the French press technique, hydrodynamic shearing, homogenization using,  
35 for example, a colloid mill or Gaulin homogenizer, and microfluidization techniques.  
Microfluidization is one presently preferred method. Other techniques involving sonication are  
also preferred.

Microfluidization is described, for example, in U.S. Patent No. 4,533,254 to Cook, et al.,

1 which is incorporated herein by reference. In a preferred microfluidization procedure, the  
liposomal emulsion is forced at high pressure through a small diameter opening and splattered  
onto a wall and then collected. In sonication techniques, the raw materials for the liposomes,  
e.g., phospholipids, are combined with chemical agents, placed in a sonicator, and sonicated for  
5 a time, at a temperature and at a speed sufficient to obtain liposomes of the desired size.

In a preferred method, the liposomes, prior to administration, are treated to protect them  
against pH changes, micellization, lipases, and digestive enzymes as they pass through the  
stomach and enter the small intestine. Preferably the liposomes are lyophilized. Alternatively,  
the phospholipid (or any other constituent of lipid wall) can be treated with an additive, such as  
10 an enteric additive or a crosslinking agent, prior to formation of the liposome. The treated  
liposomes can then be packaged in a suitable form, such as a pill or capsule, for oral ingestion.

Lyophilization may be accomplished by any method known in the art. Such procedures  
are disclosed, for example, in U.S. Patent No. 4,880,836 to Janoff, et al., the disclosure of which  
is incorporated herein by reference. Lyophilization procedures preferably include the addition  
15 of a drying protectant to the liposome suspension. The drying protectant stabilizes the liposome  
suspension. The drying protectant stabilizes the liposomes so that the size and content are  
maintained during the drying procedure and through rehydration. Preferred drying agents are  
saccharide sugars including dextrose, sucrose, maltose, manose, galactose, raffinose, trehalose  
lactose, and triose sugars which are preferably added in amounts of about 5% to about 20% and  
20 preferably about 10% by weight of the aqueous phase of the liposomal suspension. Dextrose,  
sucrose and maltose are presently preferred. Mannitol may be used in conjunction with any of the  
saccharides. Additional preservatives such as BHT or EDTA, urea, albumin, dextran or  
polyvinyl alcohol may also be used.

Alternatively, or additionally, the liposomes containing the chemical agents may be  
25 packaged for oral administration in either a pill form or a capsule. An enteric coating is  
preferably applied to the liposomes containing the chemical agents to prevent breakdown in the  
stomach. The enteric coating may be made of any suitable composition. Suitable enteric  
coatings are described, for example, in U.S. Patent Nos. 4,311,833 to Namikoshi, et al.;  
4,377,568 to Chopra; 4,385,078 to Onda, et al.; 4,457,907 to Porter; 4,462,839 to McGinley, et  
30 al.; 4,518,433 to McGinley, et al.; 4,556,552 to Porter, et al.; 4,606,909 to Bechgaard, et al.;  
4,615,885 to Nakagame, et al.; and 4,670,287 to Tsuji, all of which are incorporated herein by  
reference. Preferred enteric coating compositions include alkyl and hydroxyalkyl celluloses and  
their aliphatic esters, e.g., methylcellulose, ethylcellulose, hydroxyethylcellulose,  
hydroxypropylcellulose, hydroxybutylcellulose, hydroxyethylethylcellulose,  
35 hydroxypropylmethylcellulose, hydroxybutylmethylcellulose, hydroxypropylcellulose phthalate,  
hydroxypropylmethylcellulose phthalate and hydroxypropylmethylcellulose acetate succinate;  
carboxyalkylcelluloses and their salts, e.g., carboxymethylethylcellulose; cellulose acetate  
phthalate; polycarboxymethylene and its salts and derivatives; polyvinylalcohol and its esters,

1 polycarboxymethylene copolymer with sodium formaldehyde carboxylate; acrylic polymers and  
copolymers, e.g., methacrylic acid-methyl methacrylic acid copolymer and methacrylic  
acid-methyl acrylate copolymer; edible oils such as peanut oil, palm oil, olive oil and  
5 hydrogenated vegetable oils; polyvinylpyrrolidone; polyethyleneglycol and its esters, e.g., and  
natural products such as shellac. Other preferred enteric coatings include polyvinylacetate esters,  
e.g., polyvinyl acetate phthalate; alkylene glycolether esters of copolymers such as partial  
ethylene glycol monomethylether ester of ethylacrylate-maleic anhydride copolymer or  
diethyleneglycol monomethylether ester of methylacrylate- maleic anhydride copolymer,  
10 N-butylacrylate-maleic anhydride copolymer, isobutylacrylate-maleic anhydride copolymer or  
ethylacrylate-maleic anhydride copolymer; and polypeptides resistant to degradation in the  
gastric environment, e.g., polyarginine and polylysine. Mixtures of two or more of the above  
compounds may be used as desired.

The enteric coating material may be mixed with various excipients including plasticizers  
such as triethyl citrate, acetyl triethyl citrate, diethyl phthalate, dibutyl phthalate, dibutyl  
15 sebacate, dibutyl tartrate, dibutyl maleate, dibutyl succinate and diethyl succinate and inert fillers  
such as chalk or pigments. The composition and thickness of the enteric coating may be selected  
to dissolve immediately upon contact with the digestive juice of the intestine. Alternatively, the  
composition and thickness of the enteric coating may be selected to be a time-release coating  
which dissolves over a selected period of time, as is well known in the art.

20 Methods for preparing and coating lyophilized liposomes are described in U.S. Patent  
Application No. 08/920,374, entitled "Method for Inducing a Systemic Immune Response to an  
Antigen", the entire disclosure of which is incorporated herein by reference.

In another embodiment, the invention is directed to a method for introducing a chemical  
agent into a channel created in an organ or the skin of a patient. Liposomes containing the  
25 chemical agent are administered to the patient. The liposomes have a mean diameter of less than  
about 200 nm, preferably less than about 100 nm, still more preferably less than about 80 nm,  
and even more preferably less than about 50 nm, as determined by negative-staining transmission  
electron microscopy. The liposomes preferably have a mean diameter of at least about 20 nm.

Methods for creating microscopic channels in organs or the skin are well known to those  
30 skilled in the art. For example, a laser dermal perforator and a technique for using the same are  
described in U.S. Patent No. 5,908,416, the entire disclosure of which is incorporated herein by  
reference. A method for creating channels in the heart, for example, by transmural laser  
revascularization is described by Frazier et al. in Texas Heart Institute Journal, Vol. 25. No. 1,  
pages 24-29 (1998), the entire disclosure of which is incorporated herein by reference. A similar  
35 technique is disclosed in U.S. Patent No. 5,840,059, the entire disclosure of which is  
incorporated herein by reference. A technique for intra-operative myocardial revascularization  
is disclosed in U.S. Patent No. 5,554,152, the entire disclosure of which is also incorporated  
herein by reference.

1 After the channels are created, the chemical agent is introduced into the channels in small liposomes, i.e., liposomes having a mean diameter of less than about 200 nm, preferably less than about 100 nm, still more preferably less than about 80 nm, and even more preferably less than about 50 nm, as determined by negative-staining transmission electron microscopy. In one  
5 embodiment, the liposomes are orally administered. In an alternative embodiment, the liposomes are administered by injection. When the liposomes are administered by injection, they can be administered, for example, in the arteries upstream from the organ of interest or in the venous system downstream from the organ of interest.

10 In a particularly preferred embodiment, an attractant is introduced into the channels as they are formed. The attractant is a second chemical agent that is capable of attracting the chemical agent that is to be introduced into the channels. For example, when the chemical agent to be introduced is an antibody, the attractant can be an antigen. When the chemical agent is a negatively charged molecule, the attractant can be a positively charged molecule capable of attracting the negatively charged molecule, and vice versa. The liposomes containing the  
15 chemical agent are then introduced into the systemic circulation and bloodstream of the patient by oral administration. When the liposomes reach the organ of interest, i.e., the organ having the channels containing the attractant, the attractant causes the liposomes to enter the channels.

#### Example

20 Four-week old male CD-1 mice (16-18 g) were obtained from Charles Rivers Farms, Wilmington, Massachusetts.

Liposomes were prepared as follows: A solution of PyS DHPE was prepared in a test tube by dissolving 25 mg of PyS DHPE in 0.1 ml of chloroform. Lipid solution was then prepared in a separate test tube by combining 313 mg of DPPC, 72 mg of cholesterol, 14 mg of  
25 dicetylphosphate, 144  $\mu$ L PyS DHPE solution, and 1.056 ml of chloroform, for a total volume of approximately 1.20 ml.

360  $\mu$ l of lipid solution was aliquoted into three glass tubes. The solvent in each tube was evaporated to dryness with nitrogen gas. A maltose solution was prepared by dissolving 200 mg of maltose in 2.0 ml of water. To the maltose solution was added a chemical agent (either  
30 apomorphine or a ioxaglat, a contrast agent) or nothing (as a control). 300  $\mu$ l of maltose solution (with or without the chemical agent) was added to the lipid tubes. Q.S. with water to 9.0 ml. The chemical agents were added so that the total amount of apomorphine in the lipid solution was about 1% by weight and the total amount of ioxaglat in the lipid solution was about 10% by weight.

35 To make liposomes having a mean diameter of approximately 50 nm, the solutions were passed through a microfluidizer up to twenty times. The solutions were aliquotted into ten vials, which were placed in a freezer at 10°C overnight and subsequently lyophilized.

10 mice were orally fed approximately 1,000,000 liposomes in 10 nM sodium acetate



1 (about 110 µg) by gavage tube. After 3 days, the mice were sacrificed, and the brains, neck  
lymph nodes, hearts, spleens, Peyer's patches, livers and small intestines harvested. The tissues  
were diced into sections less than about 1 mm by a straight-edge razor, fixed in 4%  
5 glutaraldehyde for 3 hours, and then washed four times in 2M phosphate buffer. The fixed  
samples were embedded in epoxy resin, cut by microtome, placed onto 400 mesh copper grids  
and examined under an electron microscope. Liposome size was verified by negative-staining  
electron microscopy. The liposomes were generally within the range of about 28 to about 95 nm,  
with approximately 95% of the liposomes within one standard deviation of 50 nm with 500 grids  
measured after the liposomes were lyophilized and the sizes measured.

10 No liposomes were seen in the small bowel, Peyer's patches or spleen, suggesting that  
they were too small to be taken up by the mononuclear and polymorphonuclear cells and had  
passed through the venule system of the Peyer's patches into the systemic circulation.  
Liposomes were seen in the other tissue, although the slides of the brain tissue were somewhat  
unclear. The highest concentration of liposomes was in the liver. Figure 1 shows 50 nm  
15 liposomes in cervical lymph tissue, and Figure 2 shows 50 nm liposomes in the liver. The  
concentrations of liposomes observed were generally the same regardless of whether the  
liposomes contained apomorphine, contrast agent, or nothing, suggesting that the size of the  
liposome and not its contents determined the ability of the liposome to enter the systemic  
circulation.

20 The preceding description has been presented with reference to presently preferred  
embodiments of the invention. Workers skilled in the art and technology to which this  
invention pertains will appreciate that alterations and changes in the described structure may  
be practiced without meaningfully departing from the principal, spirit and scope of this  
invention. Accordingly, the foregoing description should not be read as pertaining only to the  
25 precise embodiments described, but rather should be read consistent with and as support to  
the following claims which are to have their fullest and fair scope.

## 1 CLAIMS:

5 1. A method for introducing a chemical agent into the systemic circulation of a patient comprising orally administering to the patient liposomes containing the chemical agent, wherein the liposomes have a mean diameter less than about 200 nm as measured by negative-staining electron microscopy.

2. A method according to claim 1, wherein the liposomes have a mean diameter less than about 100 nm as measured by negative-staining electron microscopy.

10 3. A method according to claim 1, wherein the liposomes have a mean diameter less than about 80 nm as measured by negative-staining electron microscopy.

4. A method according to claim 1, wherein the liposomes have a mean diameter less than about 50 nm as measured by negative-staining electron microscopy.

15 5. A method according to claim 1, wherein the chemical agent is an imaging agent.

20 6. A method according to claim 1, wherein the chemical agent is selected from the group consisting of apomorphine, growth hormone, insulin, vascular endothelial growth factors (VEGF), platelet activation factors (PAF), cytokines, endothelial adhesion factors, sulfonyleurea agents (hypoglycemia inducing), macrolide antibiotics, protein fusion agents of animal or yeast origin, slow channel inhibitors, angiotension converting enzyme inhibitors, herbs, alpha or beta sympathetic stimulating or inhibition agents, corticosteroids, male and female hormonal agents, such as estrogen, testosterone, and aldosterone, granulocyte stimulating factors, megakaryocyte  
25 stimulating agents, eicosanoids, diuretics such as furosemide, MRI and CT imaging agents, plasmids containing DNA or RNA fragments, and genes and portions thereof.

7. A method according to claim 1, wherein the liposomes are lyophilized prior to administration.

30 8. A method according to claim 1, wherein the liposomes are contained within a pill or capsule.

35 9. A method according to claim 7, wherein the liposomes are contained within a pill or capsule.

1           10. A method for introducing a chemical agent into the systemic circulation of a patient comprising orally administering to the patient lyophilized liposomes containing the chemical agent, wherein the liposomes have a mean diameter less than about 80 nm as measured by negative-staining electron microscopy.

5           11. A method for introducing a chemical agent into the systemic circulation of a patient comprising orally administering to the patient liposomes containing the chemical agent, wherein the liposomes have a mean diameter less than about 200 nm as measured by negative-staining electron microscopy, whereby the liposomes are introduced into the patient's venous circulation  
10 from the patient's Peyer's patches.

          12. A method according to claim 11, wherein the liposomes have a mean diameter less than about 100 nm as measured by negative-staining electron microscopy.

15          13. A method according to claim 11, wherein the liposomes have a mean diameter less than about 80 nm as measured by negative-staining electron microscopy.

          14. A method according to claim 11, wherein the liposomes have a mean diameter less than about 50 nm as measured by negative-staining electron microscopy.

20          15. A method according to claim 11, wherein the liposomes are lyophilized prior to administration.

          16. A method according to claim 11, wherein the liposomes are contained within a pill  
25 or capsule.

          17. A method according to claim 15, wherein the liposomes are contained within a pill or capsule.

30          18. A method according to claim 1, further comprising, prior to administration of the liposomes, creating a channel in an organ or the skin of the patient, whereby the liposomes enter the channel.

          19. A method according to claim 1, further comprising, prior to administration of the  
35 liposomes, creating a channel in the heart of the patient, whereby the liposomes enter the channel.

1           20. A method for introducing a chemical agent into a channel created in an organ or  
the skin of a patient, comprising orally administering to the patient liposomes containing the  
chemical agent, wherein the liposomes have a mean diameter of less than about 200 nm, whereby  
the liposomes are introduced into the systemic circulation and enter the channel in the organ or  
5 skin.

21. A method according to claim 20, wherein the liposomes have a mean diameter less  
than about 100 nm as measured by negative-staining electron microscopy.

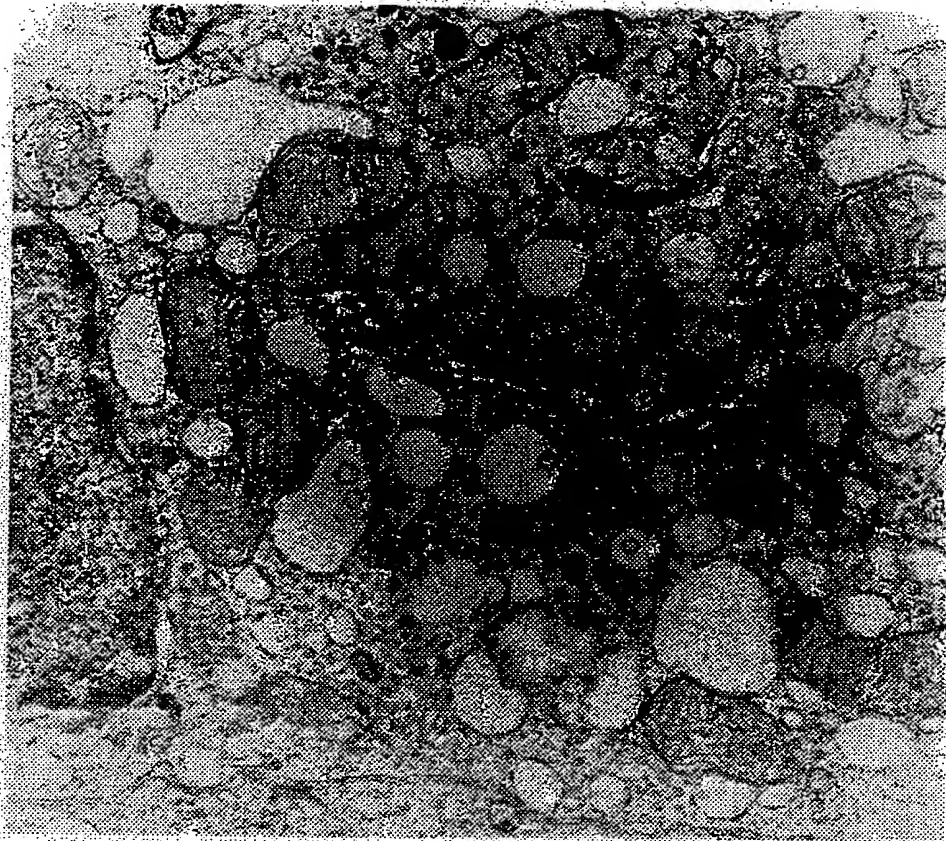
10           22. A method according to claim 20, wherein the liposomes have a mean diameter less  
than about 80 nm as measured by negative-staining electron microscopy.

23. A method according to claim 20, wherein the liposomes have a mean diameter less  
than about 50 nm as measured by negative-staining electron microscopy.

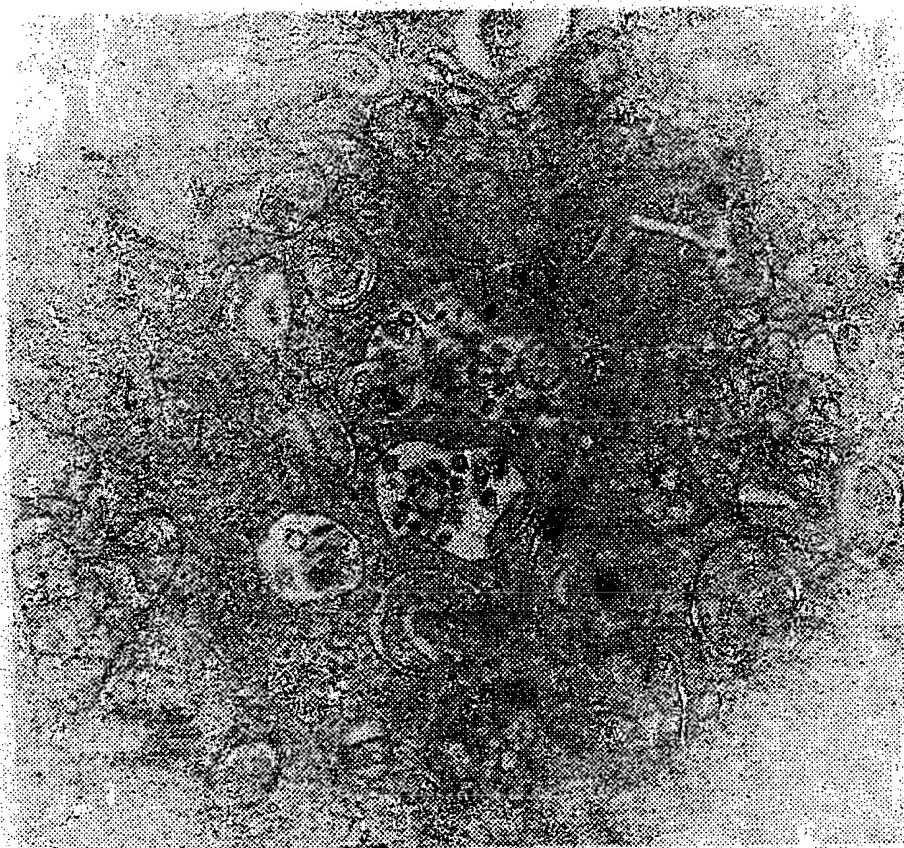
15           24. A method according to claim 20, wherein the liposomes are lyophilized prior to  
administration.

20           25. A method according to claim 24, wherein the liposomes have a mean diameter less  
than about 80 nm as measured by negative-staining electron microscopy.

*Fig. 1*



*Fig. 2*



## INTERNATIONAL SEARCH REPORT

International application No.  
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**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :A61K 9/127, 9/133, 9/20, 9/48, 51/12

US CL :425/450, 1.21, 9.321, 9.51; 935/54

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 425/450, 1.21, 9.321, 9.51; 935/54

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

Search term: liposome?, oral?, pills, tablets, capsules

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	ARAMAKI, Y. et al. Stability of Liposomes in vitro and Their uptake by Rat Peyer's Patches Following Oral Administration. Pharmaceutical Research. 1993, Vol. 10, No. 8, pages 1228-1231, especially pages 1228 and 1229.	1 & 11 ----- 2-10 & 12-25
X -- Y	US 5,059,421 A (LOUGHREY et al) 22 October 1991, abstract, columns 7-17, Examples 9-10.	1-17 ----- 18-24
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Y	US 5,554,152 A (AITA et al) 10 September 1996, abstract.	18-24

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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